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14. ABSTRACT Treatment of patients with advanced stages of breast cancer remains an unresolved clinical problem. The main objectives of this study are to determine whether immunotherapy sensitizes tumor to chemotherapy and to identify some of the main mechanisms of this effect. We investigated the possibility of a direct synergy between immunotherapy and chemotherapy <i>in vitro</i> . We found that pre-treatment of tumor target cells with doxorubicin or paclitaxel significantly increased cytotoxic effect of T-lymphocytes. Importantly, that effect was antigen-specific, since it was observed only in tumor cells loaded with specific but not a control peptide. In contrast, pre-treatment of splenocytes did not result in enhancement of target cell killing. In parallel experiments we have determined that both drugs increased the expression of p53 in tumor cells. However, that increase observed only after 48 hr of treatment and therefore could not contribute to observed sensitization of tumor cells to CTLs. To determine the effect of the combined treatment <i>in vivo</i> , mammary carcinoma TUBO was established s.c. in BALB/c mice. Dendritic cell vaccine alone slowed down tumor growth, which was consistent with previous results obtained by many laboratories. Paclitaxel had similar effect. However, in both cases tumor growth resumed in about a week after end of the treatment. In a sharp contrast, tumor size was substantially reduced in mice treated with combination of DC vaccine and chemotherapy. Most of the mice rejected tumor. Thus, this data indicates that a direct combination of chemotherapy with cancer vaccine provides substantial antitumor effect via sensitization of tumor cells to CTLs. These experimental models will be used for investigation the mechanisms of this phenomenon.				
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Introduction

Treatment of locally advanced or metastatic breast cancer remains a very difficult clinical problem. Chemotherapy is the treatment of choice for most of those patients. It is given as an adjuvant or neo-adjuvant settings alone or in combination with hormone therapy or Herceptin. Two classes of the drugs are primarily used: anthracyclines (doxorubicin, epirubicin, mitoxantrone) and taxanes (paclitaxel, docetaxel). In addition to the well-described toxicity the efficacy of the treatment remains relatively low. Median survival for patients with metastatic breast cancer is 18-24 months. Among patients treated with systemic chemotherapy 16.6% achieved complete responses and only 3.1% remained in complete remission for more than 5 years (1). Patients with locally advanced breast cancer (LABC) have a poor prognosis when treated with surgery and radiotherapy. Preoperative (neo-adjuvant) chemotherapy has been developed as an alternative therapeutic strategy as it allows surgical intervention in patients who present with bulky primary disease. In general, neo-adjuvant chemotherapy results in a complete-response rate of 10% to 35%. However, the five-year overall survival rate is only 5% to 20% (2). All these results compel the development of new approaches to therapy of breast cancer. Immunotherapy of breast cancer has not yet delivered tangible clinical results. Although some clinical trials performed in recent years demonstrated encouraging results, most of the trials showed rather limited clinical response(3). It appears that tumor escape mechanisms prevent effective recognition and elimination of tumors. New approaches are necessary to make cancer immunotherapy clinically effective. One of the most attractive approaches to cancer therapy is a combined modality treatment. However, the well-known immunosuppressive effect of chemotherapy has established a widely accepted notion that the direct combination of chemotherapy and immunotherapy will be ineffective due to the negative effect of chemotherapy on the immune system. Recently we and others have reported findings from clinical trials, which may challenge that paradigm. Patients with advanced stages of different types of cancer were treated with different vaccines. Direct clinical effect of those vaccines was quite limited. However, patients showed high objective clinical response rate to chemotherapy that immediately followed immunotherapy (4-8). Taken together these recent data suggest a possibility of new paradigm in cancer treatment. Immunotherapy can substantially enhance the effect of chemotherapy. It could be especially important for patients with advanced stage breast cancer. These data suggest a new paradigm that vaccination may be most effective in direct combination with chemotherapy. The main objectives of this study are to determine whether immunotherapy sensitizes tumor to chemotherapy and to identify some of the main mechanisms of this effect.

Body

The main goal of any cancer immunotherapy is to deliver tumor-specific CTLs to tumor sites with the prospect that CTLs will recognize and eliminate tumor cells expressing specific antigen(9). In this study we used several ways to generate tumor-specific CTLs to test the synergistic effect of immunotherapy and chemotherapy of cancer. Colon carcinoma MC38 is recognized by p53 specific CTLs due to over-expression of wild-type p53(10-12). Tumors were established s.c. and three days after tumor cell injection mice were split into treatment groups. Vaccination of mice with dendritic cells (DCs) transduced with adenovirus containing full-length mouse wild-type p53 (Ad-p53)(13) resulted in substantial delay in tumor progression (**Fig. 1a**). Treatment of mice with selected dose (12.5mg/kg i.p.) of paclitaxel (TAX) also delayed tumor growth. However, it resumed soon after the treatment was discontinued. In the combination group mice were treated with TAX three days after the second vaccination. Combination of TAX and DC vaccine demonstrated strong antitumor effect. Tumor growth remained suppressed at least for 5 weeks after start of the treatment (**Fig. 1a**). Thus, chemotherapy and immunotherapy synergized in the model where individual modality had clear antitumor activity. In the second model we used mammary carcinoma TUBO expressing Neu oncogene, which was established in Balb/c mice. Immunization was performed with DCs loaded with Neu-derived peptide. In this model selected dose of TAX had a very little antitumor activity and vaccination alone only slightly delayed tumor growth (**Fig. 1b**). However, combination of DC vaccine with TAX treatment resulted in a substantial antitumor effect in this model as well (**Fig. 1b**). To test this approach in an experimental system with adoptive transfer of T cells we used mice bearing EG-7 tumors (EL-4 cells expressing chicken ovalbumin (OVA)). T cells specific for OVA-derived peptide (SIINFEKL) were used for adoptive transfer. Both T cells and TAX alone caused substantial decrease in tumor growth. However, combination of T-cell transfer with TAX administration not only decreased tumor growth more effectively than individual treatment but also substantially delayed tumor progression (**Fig. 1c**). To evaluate the ability of T cells to penetrate into tumor parenchyma, OVA-specific T cells were labeled with fluorescent tracker CMAC and transferred into EG-7 tumor-bearing mice 3 days after TAX injection. Tumors were excised 24 hr after T-cell administration. Only a few tumor-infiltrating T cells were present in the mice not treated with TAX. In contrast in mice treated with TAX the number was significantly higher (**Fig. 1d**).

Next we investigated whether chemotherapy can affect *in vitro* cytotoxicity of CTLs. Antigen-specific T cells were generated by immunizing naïve C57BL/6 mice with Ad-p53 DCs. Splenocytes were re-stimulated with p53-derived H2K^b peptide (KYMCNSSCM) for 5 days and then used as effectors in CTL assay. As a target we used EL-4 tumor cells loaded with control or specific peptide. Tumor cells were pre-treated with TAX or doxorubicin (DOX) for 18 hr. In preliminary experiments we selected the maximal dose of the drugs that did not induce more than 5% apoptosis of tumor cells after 18 hr of culture. After 48 hr in culture the same dose caused more than 80% of tumor cell death (**data not shown**). Tumor cells were washed from the drugs, loaded with control or specific peptides, and then cultured at different ratios with effector cells. CTLs displayed moderate but clear specific cytotoxicity (**Fig. 1e**). Pretreatment of tumor cells with TAX or DOX did not result in increased cytotoxicity of target cells loaded with control peptide. However, cytotoxicity of tumor cells loaded with p53 peptide was significantly increased (**Fig. 1e**). In reverse experiments splenocytes from immunized mice stimulated with specific peptide were pre-treated for 18 hr with the same doses of TAX and DOX. After that time cells were washed from the drugs and used as effectors in CTL assay against EL-4 target cells. Pretreatment of splenocytes with TAX did not affect specific cytotoxicity, whereas DOX even reduced it (**Fig. 1f**). Thus, it appears that TAX and DOX potentiated the effect of CTLs by affecting tumor cells but not the activity of CTLs. Importantly, synergistic cytotoxic effect remained when both splenocytes and tumor cells were pre-treated with TAX (**Fig. 1g**), which would closely reflect the actual condition of treatment with chemotherapy. Similar results were obtained in experimental models with CTLs specific for Neu-derived peptide and 4T1 tumor cells transfected with Neu expressing plasmid (**Fig. 1h**) as well as with 2C transgenic T cells that recognize SIYRYGL peptide (**Fig. 1i**). Thus, these data strongly indicate that chemotherapy sensitizes tumor cells to the cytotoxic effect of CTLs.

Mechanism of synergy between chemotherapy and CTLs

To investigate the kinetic of interaction between CTLs and tumor cells in real time we used transgenic CD8⁺ T cells isolated from OT-1 mice expressing TCR specific for OVA-derived peptide SIINFEKL. OT-1 T cells were labeled with CFDA SE cell tracer and mixed at 10:1 ratio with target EL-4 cells loaded with control or specific peptides in the presence of propidium iodide (PI). Cells were observed using live cell imaging. One hour incubation was not sufficient for CTLs to kill non-treated tumor cells loaded with specific peptide. However, if EL-4 cells were pre-treated with TAX, cytotoxic effect was easily detectable. This fast kinetic was confirmed in

chromium release assay and apoptosis assay using Annexin-V/7-AAD staining (**Fig. 2a**). In contrast to non-treated target cells EL-4 cells pre-treated with TAX were susceptible to apoptosis induced by specific CTLs as early as 30 min of incubation. This effect became prominent after 90 min of incubation. The proportion of apoptosis among tumor cells pre-treated with TAX and loaded with specific peptide doubled than that of tumor cells that were not treated with TAX or not loaded with specific peptide (**Fig. 2a**). Similar effect was observed when EL-4 target cells were pre-treated with DOX or cisplatin (CIS) (**data not shown**).

Next we evaluated the mechanism of apoptosis caused by combination of chemotherapy and CTLs. Pretreatment of tumor cells with TAX caused substantial increase in cleaved caspase 3 in tumor cells. However, this effect was not enhanced by the addition of CTLs (**Fig. 2b**). In contrast, CTLs substantially increase the proportion of cytochrome C positive tumor cells. TAX, however did not enhance this effect of CTLs (**Fig. 2c**). Thus, it appears that TAX and CTLs affect different components of apoptotic pathway during initial interaction between CTLs and tumor cells.

CTLs mediate their cytotoxic effect via two major pathways: Fas-FasL and perforin-granzyme B (GrzB). Treatment of EL-4 tumor or MC38 tumor cells as well as splenocytes with selected doses of TAX, CIS, or DOX did not significantly change the level of expression of Fas or FasL (**data not shown**) suggesting that it is unlikely that chemotherapy sensitizes tumor cells to CTLs via up-regulation of these molecules. To assess the role of GrzB, EL-4 cells pre-treated with TAX were loaded with control or specific peptides and labeled with cell tracker blue CMAC. Tumor cells were incubated with OT-1 CD8⁺ CTLs at 1:10 ratio followed by staining with anti-GrzB antibody. Very few GrzB positive tumor cells were seen when CTLs were cultured for 15 min with untreated EL-4 cells or TAX pre-treated EL-4 cells loaded with control peptide. After 7 min of culture the proportion of GrzB positive tumor cells was more than 3-fold higher when tumor cells were pre-treated with TAX and loaded with specific peptide (**Fig. 3a**). These results suggested that pre-treatment of tumor cells with TAX allowed fast penetration of GrzB into tumor cells. To verify these observations we used recombinant mouse GrzB, which was modified to block its protease activity and thus does not cause apoptosis of the cells. EL-4 cells were pre-treated with TAX, DOX or CIS and then incubated with recombinant GrzB for 30 min. Cells were then stained with anti-GrzB antibody. On comparison with non-treated cells treatment of tumor cells with all three drugs resulted in a substantial increase in the intracellular GrzB level (**Fig. 3b,c**). When GrzB activity was blocked with specific inhibitor Z-AAD-CMK it abrogated the effect of TAX on CTL-inducible apoptosis of tumor cells (**Fig. 3d**) confirming the critical role of this mechanism in the synergistic effect of chemotherapy and CTLs.

Mechanism of synergistic antitumor activity of CTLs and chemotherapy

Our data demonstrated that chemotherapeutic drugs increase tumor cell permeability to GrzB. GrzB is released by activated CTLs during antigen-specific interaction with tumor cells. However, during this interaction penetration of GrzB to tumor cells depends on perforin. We hypothesized that chemotherapy could cause a “by-stander” CTL activity, whereby GrzB released by activated CTLs were able to penetrate tumor cells that do not express specific antigen and thus normally are resistant to GrzB. To test this hypothesis we incubated effector OT-1 T cells with two target cells mixed at 1:1 ratio. One was EL-4 cells loaded with specific peptide and left unlabeled, the other one was EL-4 cells loaded with control peptide and labeled with chromium. Therefore chromium release only from EL-4 cells loaded with control peptide could be detected. TAX treated EL-4 cells loaded with control peptide are not directly recognized by OT-1 CTLs. No cytotoxicity was detected when target cells were not treated TAX. However, pre-treatment of target cells with TAX resulted in a substantial increase in chromium release (**Fig. 4a**). It was possible that dying peptide-loaded tumor cells could release specific peptide that would be picked up by tumor cells loaded with control peptide and make them more sensitive to CTL recognition. To exclude this possibility we used two models. Neu-specific T cells were incubated with chromium labeled 4T1 tumor cells (that don't express Neu antigens) and unlabeled 4T1 cells expressing transfected Neu protein. Pre-treatment of tumor cells with TAX substantially increased chromium release from antigen-negative target cells (**Fig. 4b**) that otherwise, are not recognized by CTLs (**Fig. 1h**). Finally, we used an experimental system with unlabeled wild-type melanoma B16F10SK cells and chromium labeled B16F10K^b cells that are deficient for H2K^b molecule and therefore cannot present H2K^b restricted peptide. CTLs generated against H2K^b epitope derived from tyrosinase related protein 2 (TRP-2) were used as effector cells. Very little cytotoxicity was detected when target tumor cells were not pre-treated with TAX. However, pre-treatment with TAX dramatically increased the cytotoxicity of H2K^b negative cells that cannot be recognized directly by TRP-2 specific CTLs (**Fig. 4c**).

Thus, pre-treatment of tumor cells with TAX allowed CTLs to by-pass the requirement for specific recognition to exert cytotoxic effect. If this is indeed the case then pre-treatment of tumor cells with TAX should eliminate

the requirement for perforin in CTL activity. To test this hypothesis SIINFEKL-specific CTLs were generated by immunization of control and perforin knockout (KO) mice with the peptide and then used as effectors against EL-4 tumor cells loaded with control and specific peptide. As expected CTLs did not recognize EL-4 cells loaded with control peptide and in the absence of CTL activation no cytotoxicity was detected against TAX treated target as well (**Fig. 4d**). However, wild-type CTLs recognized and killed EL-4 target cells loaded with specific peptide (**Fig. 4e**). No specific cytotoxicity was detected in perforin KO CTLs if targets were untreated. However, pre-treatment of EL-4 cells with TAX resulted in a substantial level of specific cytotoxicity comparable with the level of wild-type CTLs (**Fig. 4e**). To verify the possible role of this mechanism *in vivo*, we performed adoptive transfer experiment where EG-7 tumor-bearing mice were treated with adoptive transfer of T cells from mice immunized with SIINFEKL. Perforin KO mice demonstrated high level of antigen-specific CD8⁺ T cells generated in response to immunization. However, these CTLs failed to provide any antitumor effect after adoptive transfer to untreated tumor-bearing host. In contrast, the same perforin KO CTLs showed substantial antitumor effect when they were transferred into TAX treated tumor-bearing mice (**Fig. 4f**).

Our data suggest a novel model of combined effect of CTLs and chemotherapy. When used as single modality cancer vaccines or T-cell transfers only limited numbers of T cells are able to penetrate tumor parenchyma. CTLs exert cytotoxic effect only against tumor cells that express specific antigen, since it requires direct cell-cell contact. This effect is limited by the expression of specific antigen on tumor cells and the presence of different immune suppressive factors in the tumor microenvironment(14). Chemotherapy causes disruption of tumor stroma that allows better penetration of antigen-specific T cells. Most importantly chemotherapy may affect the fluidity of the tumor cell membrane(15, 16), which may result in increased permeability of the cells to GrzB. In this situation a small number of activated CTLs able to release GrzB can kill neighboring tumor cells without cell-cell contact. Therefore even tumor cells that do not express specific antigen would be susceptible to the effect of CTLs (**Fig. 4g**). It is likely that this effect would not be permanent and subject to the elimination of CTLs by chemotherapy and immune suppressive tumor microenvironment. However, it may provide sufficient window to achieve antitumor effect. Since memory T cells are more resistant to chemotherapy than the effector T cells(17), it is possible that subsequent immunization would be able to boost antitumor immunity and thus provide a long lasting effect.

Figure legends

Figure 1. Synergy between chemotherapy and immunotherapy *in vivo*

- a. Murine colon carcinoma tumor was established in C57BL/6 mice by sub-cutaneous (s.c) injection of MC38 tumor cells. Treatment was started 5 days after tumor inoculation. Mice in treatment groups (DC, DC + TAX) were injected s. c with 5×10^5 DCs transduced with recombinant adenovirus containing mouse wild-type p53 gene. Immunizations were repeated twice at 7-day intervals. Treatment with TAX (12.5mg/kg) was started three days after the second immunization. Tumor size was measured and presented as a product of two longest dimensions. Each group included 5 mice and Mean \pm SE are shown.
- b. Mammary carcinoma TUBO was established s.c. in Balb/c mice. The treatment times and intervals were same as in Fig 1a. DCs used for immunizations were loaded with 10 μ g/ml of Neu-derived peptide. Each group included 5 mice.
- c. T cells from mice immunized with OVA-derived peptide SIINFEKL were transferred to EG7 tumor bearing C57BL/6 mice by i.v injection of 5×10^6 cells. The treatment protocol for the treatment with TAX and adoptive transfer has been described in Methods. Each group included 4 mice.
- d. EG7 tumors were established s.c. in C57BL/6 mice. On day 16, the mice were treated with TAX (12.5mg/kg b.w) i.p. Three days later they were administered with 5×10^6 cell tracker blue CMAC labeled purified T cells from mice immunized with SIINFEKL. The tumors were removed 24 hr later and cryosections were prepared. The slides were observed under a Leica fluorescence microscope and 20 high power (x 400) fields were counted per slide. The histogram represents the number of T cells per tumor field in three mice per group. Mean \pm SD. are shown. $p < 0.05$ in two-sided Student's t-test.
- e. Tumor-free C57BL/6 mice were immunized with K^b bound p53-derived peptide. Splenocytes were isolated, re-stimulated with the specific peptide for 6 days and then used as effectors in CTL assay against EL-4 target cells loaded with specific (p53) or control peptides. EL-4 cells were pretreated overnight with either 1.5 μ g/ml doxorubicin (DOX) or 12.5 nM paclitaxel (TAX). Standard 4-hr ⁵¹Cr-release assay was performed. Each experiment was performed three times with the same results.
- f. Splenocytes from immunized mice described in Fig 1e were pretreated overnight with either DOX or TAX at doses described above and used as effectors against EL-4 target cells loaded with specific or control peptides in a Cr⁵¹ release assay.
- g. This figure represents results of a CTL assay wherein both effectors (splenocytes from mice immunized with K^b bound p53-derived peptide) and targets (EL-4 cells loaded with specific peptide) were pretreated with TAX as described above.
- h. T cells from mice immunized with Neu-derived peptide were used as effectors against either non-modified 4T1 cells or 4T1 cells transfected with Neu expressing plasmid. The targets were pre-treated with TAX for 18 hr.
- i. T cells from 2C transgenic mice were used as effectors against EL-4 cells loaded with the specific (S.P.) or control (C.P.) peptides. The target cells were pre-treated with TAX overnight.

Figure 2. Kinetic of apoptosis in combination therapy

- a. For the detection of early apoptosis the effector OT-1 T cells were labeled with DDAO-SE and incubated with target cells EL-4 cells loaded either with specific (S.P.) or control (C.P.) peptides at a 20:1 ratio. After the indicated incubation time cells were stained with Annexin-V-FITC and 7-AAD. The proportion of Annexin-V positive cells was measured within the population of tumor cells by flow cytometry. Typical result of one of three performed experiments is shown.
- b. Cleaved caspase 3 in tumor cells. Untreated and TAX treated EL4 targets were labeled with DDAO-SE and loaded with control (C.P.) or specific (S.P.) peptide. The tumor cells were incubated with purified OT-1 T cells at 1:10 ratio. After indicated incubation time cells were permeabilized and labeled with PE conjugated antibody against cleaved caspase 3. Target cells were gated and the levels of cleaved caspase 3 in the target cells was analyzed by flow cytometry. Typical example of one experiment and cumulative results of five performed experiments are shown. * - statistically significant differences from untreated EL-4 cells.
- c. Cytochrome C in tumor cells. Experiments were performed as described in Fig. 2b. The cells were permeabilized, fixed and labeled with anti- cytochrome C antibody. Typical example of one experiment and cumulative results of five performed experiments are shown. * - statistically significant differences from untreated EL-4 cells.

Figure 3. The effect of chemotherapy on permeability of tumor cells to granzyme B

- a. EL-4 cells were treated with TAX and loaded with either control (C.P.) or specific (S.P.) peptides as described above. Cells were labeled with tracker blue dye (CMAC) and mixed with OT-1 cells in a 1:10 ratio and incubated for 7 and 15 min, then fixed, and stained with anti-GrzB monoclonal antibody followed by FITC

conjugated secondary antibody. The proportion of GrzB positive cells among blue target cells was calculated in triplicates by counting 200 target cells. * - statistically significant ($p < 0.05$) difference from untreated tumor cells loaded with control peptide.

b. CMAC labeled TAX treated EL-4 cells were incubated with 1 μ g/ml recombinant mouse GrzB for 30min. Cells were fixed and stained with anti-mouse GrzB antibody. The presence of GrzB was detected by flow cytometry.

c. The above experimental procedure was used for detecting the presence of GrzB positive cells in CIS and DOX treated EL-4 cells. Histogram overlays represent non-treated EL-4 cells (red line) and CIS or DOX treated cells (blue line). Each experiment was repeated at least twice with the same results.

d. OT-1 T cells were pre-treated with GrzB inhibitor I (Z-AAD-CMK) prior to incubation with EL-4 cells. The target cells were labeled with CMAC while the effectors were labeled with DDAO-SE. The target population was assessed for apoptosis using Annexin-V-FITC - 7AAD staining and analyzed using flow cytometry. Apoptosis was measured among tumor cells. Data represents two experiments

Figure 4. Mechanism of synergistic antitumor activity of CTLs and chemotherapy

a. EL-4 cells loaded with control peptide and labeled with ^{51}Cr were mixed at 1:1 ratio with unlabeled EL-4 cells loaded with specific peptide. Mixture of target cells was incubated with OT-1 T cells at indicated ratios. Pre-treatment of target cells with TAX was performed overnight. Standard 4-hr chromium release assay was performed in duplicates. Experiments were repeated three times with the same results. Appropriate positive controls were set up with each experiment (data not shown).

b. Experiment was performed essentially as described in Fig. 4a. As target cells chromium labeled 4T1 cells mixed with unlabeled 4T1-Neu cells were used. Effector T cells were obtained from splenocytes of BALB/c mice immunized with Neu-derived peptide as described in Methods.

c. Unlabeled wild-type B16F10 tumor cells were mixed at 1:1 ratio with ^{51}Cr labeled B16F10 cells with deleted H2K^b. These target cells were incubated with T cells isolated from mice immunized with TRP-2-derived peptide as described in Methods. Two experiments with similar results were performed.

d, e. Perforin KO and C57BL/6 mice were immunized with SIINFEKL. Purified T cells from the spleens of these mice were used as effectors in ^{51}Cr release assay. EL-4 cells loaded with control (C.P.) or specific (S.P.) were used as targets. Half of the target cells were pre-treated with TAX overnight. Two experiments with the same results were performed.

f. An adoptive transfer experiment using T cells from SIINFEKL immunized wild-type or perforin KO mice was performed as per the method described in Fig 1c. The treatment protocol has been described in Methods. Tumor size was measured and presented as described in Fig 1c.

g. Schematic representation of the advantage of combination therapy in targeting tumor cells over the use of immunotherapy alone. The figure is a depiction of the possible mechanism of bystander lysis of non-specific targets within tumors. Yellow circle – CTLs, grey – tumor cells, green – GrzB. Red bars depicts the presence of specific antigen on tumor cells.

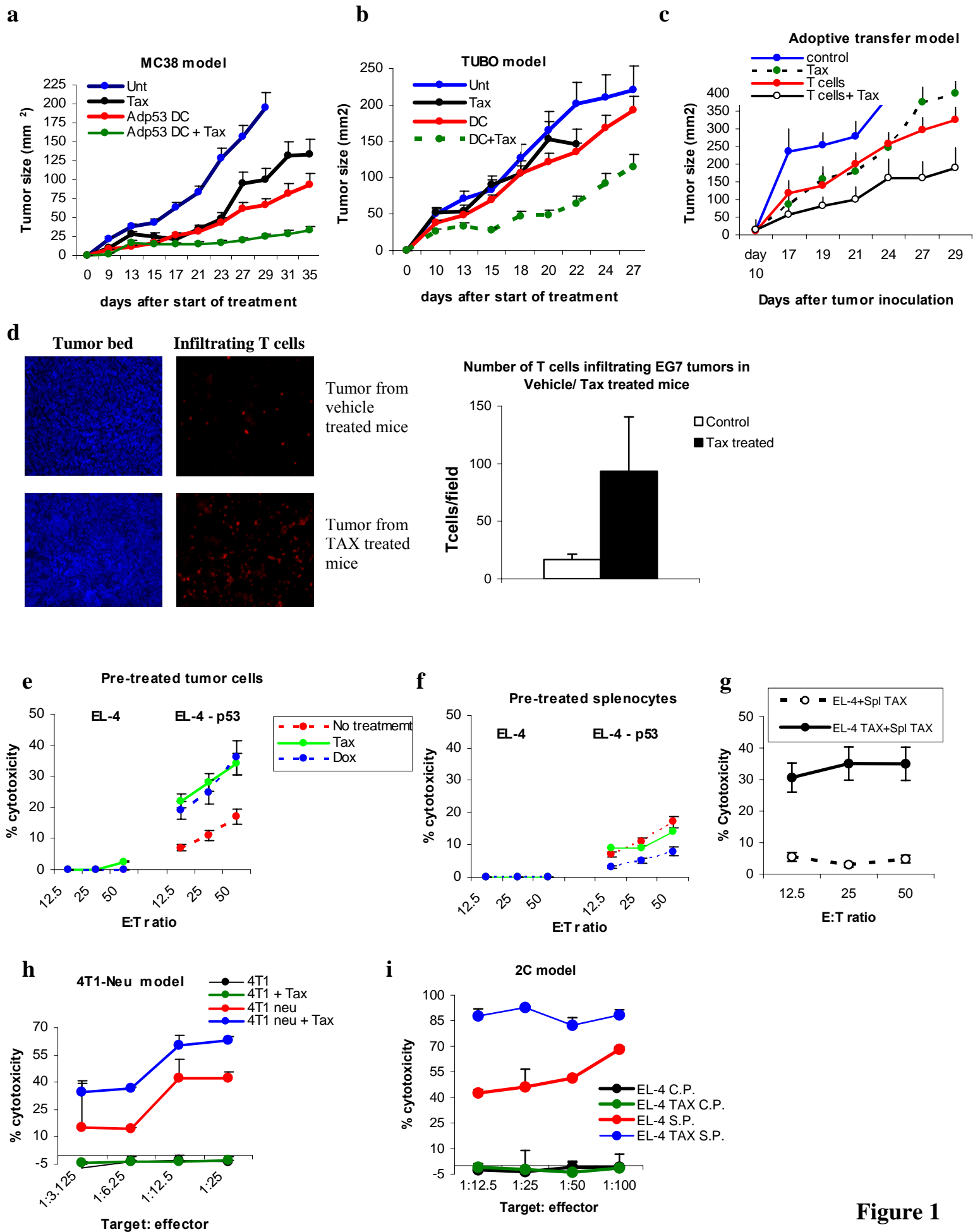
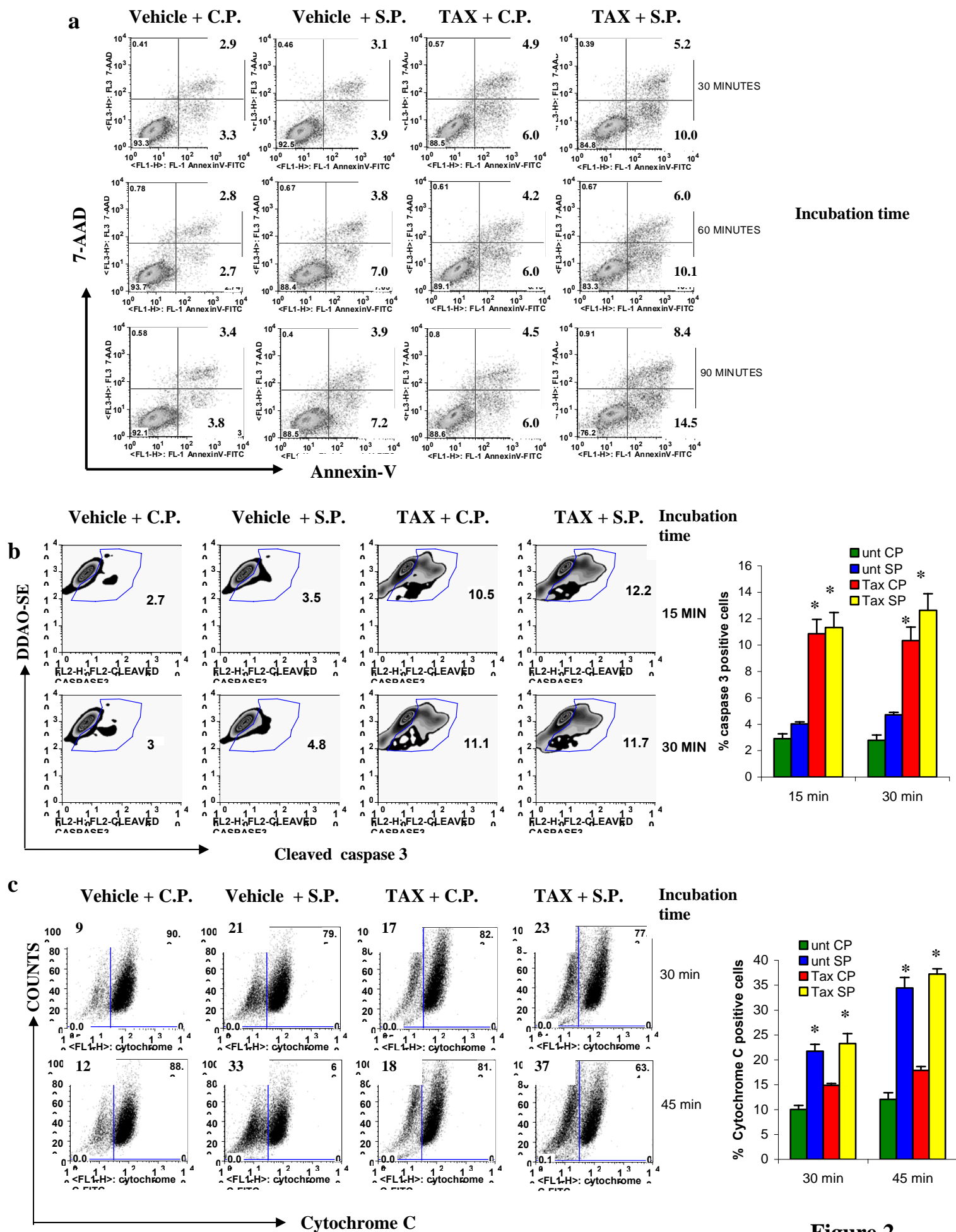


Figure 1



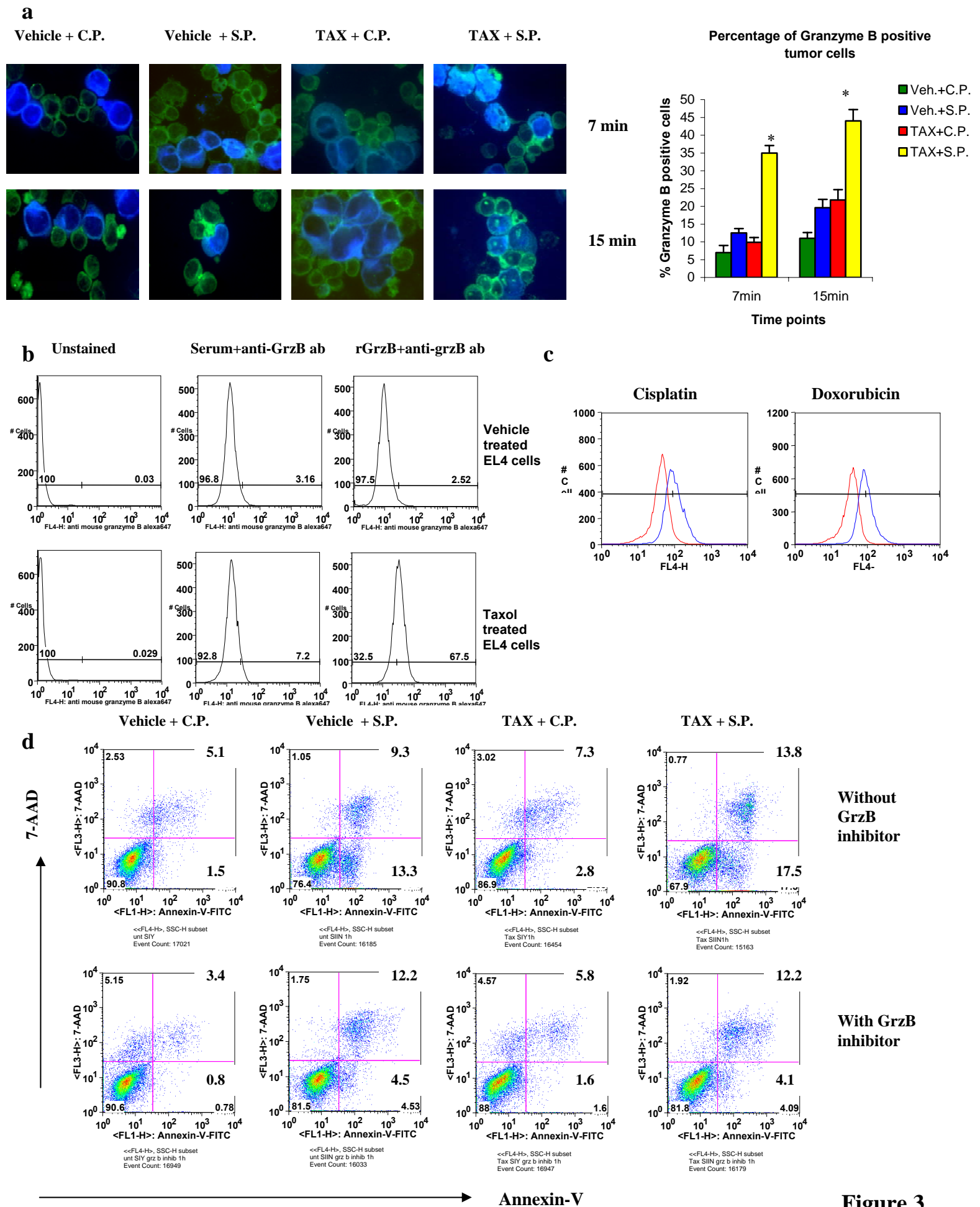


Figure 3

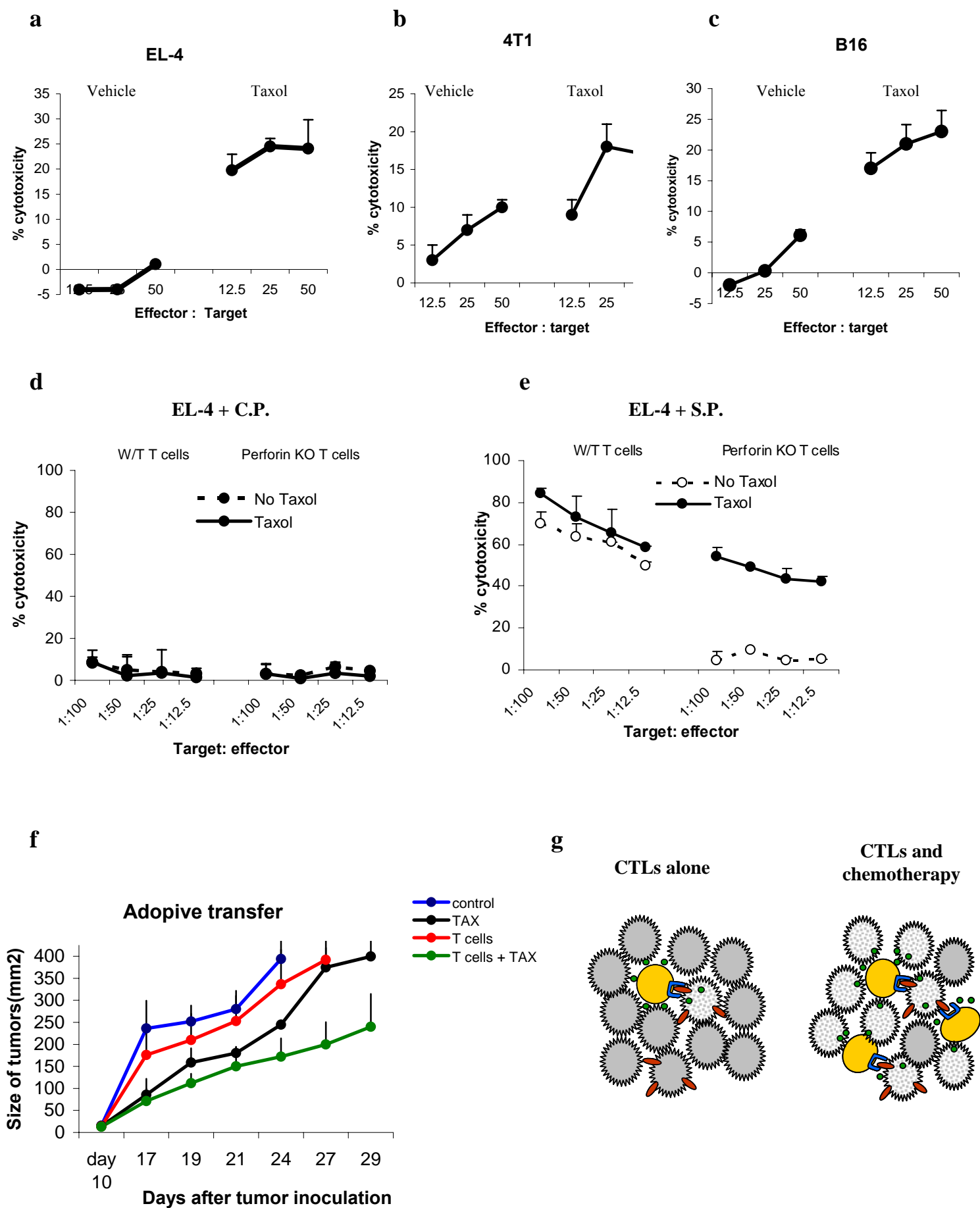


Figure 4

Key Research Accomplishments

- In experimental model of breast cancer we have determined that immunotherapy has synergized with chemotherapy in potent antitumor activity
- In experiments *in vitro* we have found that chemotherapy sensitize tumor cells to the effect of CTLs. At the same time, pre-treatment of CTLs with chemotherapeutic drugs did not improve cytotoxicity. Thus, synergistic effect of immunotherapy and chemotherapy was mediated primarily by the effect of chemotherapy on tumor cells.
- Chemotherapy and CTLs utilize different pathways in the induction of tumor cell apoptosis.
- We have identified novel mechanism of combined effect of chemotherapy and immunotherapy of cancer mediated by permeability of tumor cells to granzyme B released by CTLs.
- CTLs raised against specific antigens were able to induce apoptosis in the neighboring tumor cells that do not express those antigens. These data suggest that small number of low affinity CTLs could cause potent antitumor effect when combined with chemotherapy and provide a strong rationale for combination of these modalities in treatment of patients with advanced cancers.

Reportable Outcomes

None at this moment

Conclusions

Our data suggest a novel model of combined effect of CTLs and chemotherapy. When used as single modality cancer vaccines or T-cell transfers only limited numbers of T cells are able to penetrate tumor parenchyma. CTLs exert cytotoxic effect only against tumor cells that express specific antigen, since it requires direct cell-cell contact. This effect is limited by the expression of specific antigen on tumor cells and the presence of different immune suppressive factors in the tumor microenvironment. Chemotherapy causes disruption of tumor stroma that allows better penetration of antigen-specific T cells. Most importantly chemotherapy may affect the fluidity of the tumor cell membrane, which may result in increased permeability of the cells to GrzB. In this situation a small number of activated CTLs able to release GrzB can kill neighboring tumor cells without cell-cell contact. Therefore even tumor cells that do not express specific antigen would be susceptible to the effect of CTLs. It is likely that this effect would not be permanent and subject to the elimination of CTLs by chemotherapy and immune suppressive tumor microenvironment. However, it may provide sufficient window to achieve antitumor effect. Since memory T cells are more resistant to chemotherapy than the effector T cells, it is possible that subsequent immunization would be able to boost antitumor immunity and thus provide a long lasting effect.

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